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Abbreviated running Title: Pulses and vitamin D/iron bioaccessibility

Abstract

In this study, we evaluated vitamin D and mineral (iron, zinc, magnesium) transfer to the bolus aqueous phase during the digestion of meals with/without pulses. We performed *in vitro* digestions using test meals made either of i) beef, semolina and/or chickpeas, or of ii) potatoes supplemented or not with fibers, phytates, tannins and saponins. Chickpea presence led to a decrease in vitamin D bioaccessibility (-56%, $p \leq 0.05$) and mineral solubility (-28% for iron, $p \leq 0.05$) compared with meals with beef and/or semolina only. This effect was largely compensated for vitamin D by the fact that this vitamin was more stable during digestion of meals based on plant food only than of meals with beef. Tannins were the most deleterious compounds for iron solubility, while phytates and tannins decreased vitamin D bioaccessibility. Agronomical or technical solutions to selectively decrease the amount in pulses of compounds that affect micronutrient bioavailability should be further explored.

Key words: cholecalciferol; iron; zinc; magnesium; bioavailability; chickpeas; semolina; beef

1. Introduction

Rethinking food systems for sustainability, from production to consumption, to provide better nutritional value at lower environmental cost is a major challenge. Pulses might contribute to improve sustainability, due to restoration of soil nitrogen (Gan et al., 2015) and to their content in high-quality proteins, fibers and minerals (Margier et al., 2018). Unfortunately, pulse consumption has declined in past decades in industrialized countries (Oliveira, de Moura, & Cunha, 2019), despite the efforts by international authorities, such as the Food and Agriculture Organization (FAO), to promote their consumption. In some French traditional dishes (i.e. cassoulet) pulses are consumed asin a replacement of the meal starchy component. From the viewpoint of diet sustainability, bringing pulses, and other protein-rich plant foods, back in our plates would be of interest for substituting resource-consuming animal proteins (Salome et al., 2019).

However, pulses may also present some nutritional disadvantages, depending on their amount in the diet. Indeed, they contain several bioactive compounds, such as phytates or tannins, that persist after cooking and that can negatively affect mineral absorption by quenching them during digestion (Champ, 2002). Additionally, we experimentally demonstrated that phytates and tannins, as well as fibers and saponins, can negatively influence the bioavailability of fat-soluble vitamins (Margier et al., 2019), possibly due to modulation of lipolysis during digestion.

These observations raise several questions about the amount of pulses that might be included in the diet without reducing the meal overall nutritional quality, especially by decreasing micronutrient bioavailability.

In this work, we assessed for the first time the bioaccessibility/solubility of a fat-soluble vitamin (vitamin D_3, i.e. cholecalciferol) and minerals (iron, magnesium and zinc) in test meals made with different combinations of meat (minced beef), cereals (semolina) and pulses

(chickpeas). We then investigated the specific effect of fibers, phytates, tannins and saponins on both vitamin D and mineral solubility. Our results indicate that this *in vitro* digestion model is suitable for assessing the bioaccessibility/solubility of fat-soluble micronutrients and minerals in complex test meals.

2. Materials and methods

2.1. Chemicals

Vitamin D_3 (> 95% pure), retinyl acetate (> 95% pure), trioctanoin, sodium taurodeoxycholate, pancreatin (P7545; 8×USP), fibers (cellulose), phytates (phytic acid sodium salt hydrate), saponins (purified quillaia saponins), and tannins (grape seed oligomeric proanthocyanidins) were purchased from Sigma Aldrich (Saint-Quentin-Fallavier, France). Porcine colipase was purified as previously described (Rugani et al., 1995) and stored as a stock solution at 1mg/mL. Refined olive oil was generously provided by Dr Marie-Josèphe Amiot (UMR Moisa, Montpellier, France). Minced beef (Casino brand, 5% of fat), semolina (Casino brand) and fresh potatoes in bulk were bought at a local supermarket (Casino, Marseille, France). Chickpeas (*Cicer arietinum*) were purchased from the CIACAM society (Vitrolles, France). The chickpeas used in this study were from the same lots than those fully characterized in a previous work (Margier et al., 2018).

Fiber, phytate, saponin and tannin measurements in pulses and pureed potatoes

Fibers, phytates, saponins and tannins were previously measured in different prepared pulses (Margier et al., 2018) and potatoes (Margier et al., 2019). Fibers, phytates, saponins and tannins from semolina were measured with the same methods. Results are summarized in Table 1.

2.2. *In vitro* digestion

Minced beef was pan-cooked (no added fat), semolina was cooked in an equal volume of hot water for 10 min, and chickpeas were canned at Centre Technique de Conservation des

Produits Agricoles (CTCPA, Avignon, France), according to a standard protocol (Margier et al., 2018).

The test meals used in the first set of *in vitro* digestion experiments contained 100 µL of refined olive oil supplemented with 100 ng of vitamin D, and 0.6 g of pan-cooked minced beef, 3.35 g of pureed potatoes (control) and/or 3.35 g of canned chickpeas. The proportions of the different foods were chosen according to a previous validated protocol (Reboul et al., 2006).

The test meals used in the second set of experiments contained 3.35 g of potatoes and 200 µL of refined olive oil, and were supplemented with purified cellulose (0.35 mg), phytates (21.25 mg), saponins (3 mg) or tannins (0.2 mg). These amounts were chosen to mimic their concentration in the test meals with chickpeas (Margier et al., 2018). Each test meal was supplemented with 100 ng of vitamin D dissolved in refined olive oil, and with 80 µg of iron in the form of ferrous sulfate monohydrate (American International Chemical, Westborough, MA).

The *in vitro* digestion experiments were carried out in quadruplicate as previously described, except that all the volumes of solutions were divided by two (Malapert et al., 2018; Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi, & Borel, 2006). The final mixture representing the digesta was centrifuged (2500 g, 10 °C, 1 h) to separate the aqueous phase containing the mixed micelles and the solubilized minerals. Aliquots of digesta and aqueous phases were stored at -80°C under nitrogen atmosphere until analysis.

2.3. Mineral analysis

Iron, zinc and magnesium were extracted using a closed-vessel microwave digestion system (Ethos-1, Milestone, Italy) from about 1 g of sample (raw food/ingredients, digesta, or aqueous phases) in a 0.25:1.75 nitric acid/hydrogen peroxide mixture. The closed vessels

were placed in the microwave oven and digested at 1200 W for 30 min. After cooling, samples were diluted to 6 mL with ultrapure water. Iron, zinc and magnesium contents were analyzed with an Agilent 5100 VDV ICP-OES spectrometer (Agilent, Les Ulis, France).

Mineral solubility was calculated i) as percentage of the soluble mineral recovered in the aqueous phases relative to the total mineral recovered in the whole digesta, and ii) as percentage of the soluble mineral recovered in the aqueous phases relative to the initial amount added in the test meal at the beginning of digestion.

2.4. Vitamin D analysis

Vitamin D was extracted from 100 µL of digesta or 100µL of mixed micelles (aqueous phases) as follows. 400 µL distillated water was added to each sample to reach a final volume of 500µL. Retinyl acetate (internal standard) was added to the samples in 500µL ethanol. Then, mixtures were extracted twice with two volumes of hexane. The hexane phase obtained after centrifugation (500g, 10min, 4°C) was evaporated to dryness under nitrogen, and the dried residue was dissolved in 200µL acetonitrile/methanol (60/40, v/v). A volume of 100-180µL was used for HPLC analysis. The HPLC system included a Shimadzu separation module (LC-20ADSP HPLC Pumps and SIL-20CHT Autosampler, Shimadzu, Marne-la-vallée, France) and an SPD-M20A Shimadzu photodiode array detector (PDA, detection at 265nm for vitamin D, 325nm for retinyl acetate, spectral analysis between 190nm and 600nm). Vitamin D and retinyl acetate (Sigma Aldrich) were separated using a 250 × 4.6 nm, 5µm Zorbax Eclipse XDB-C_{18} column and a guard column (Agilent). The mobile phase was 60% acetonitrile, 38% methanol and 2% water. Flow rate was 1.5mL/min and the column was kept at a constant temperature (40°C). Vitamin D was identified by spectral analysis and/or on the basis of the retention time and co-injection compared with the pure standards. Quantification was performed using the Chromeleon software (version 6.8, ThermoFisher

Scientific, Villebon sur Yvette, France) by comparing the peak area with the standard reference curves. All HPLC-grade solvents were from Carlo-Erba (Peypin, France).

Vitamin D bioaccessibility was calculated as the percentage of vitamin D recovered in mixed micelles relative to the amount of vitamin recovered in the whole digesta at the end of the digestion. As vitamin D could be variably degraded during digestion, depending on the meal composition, vitamin D bioaccessibility was also calculated as the percentage of vitamin D recovered in mixed micelles relative to the initial amount of vitamin D added to the meal at the beginning of digestion.

2.5. Lipid extraction and analysis by thin-layer chromatography coupled with flame ionization detection

Lipolysis products were analyzed in the digesta of test meals made of semolina (control) or chickpeas according to Cavalier et al. (Cavalier et al., 2009). Briefly, lipids were extracted from 1 mL of digesta using 5 mL of a chloroform/methanol mixture (2:1, v/v) after acidification of the digesta with 200 µl of 1N HCl, followed by vortex homogenization and centrifugation. The lower organic phase was then recovered and dried by adding magnesium sulfate powder to each sample to remove the residual water that could interfere with lipid separation. Samples were again centrifuged to sediment magnesium sulfate, and the organic phase was removed for chromatographic analysis. The lipid extracts were spotted (1 µL) on SIII chromarods (Iatron laboratories, Tokyo, Japan) using a semi-automated sample spotter. Elution was carried out with a heptane/ether/formic acid mixture (55:45:1, v/v/v) for approximately 20 min. The chromarods were then dried and solvent evaporated in an oven at 150 ° C for 15 min. The detection was carried out using a Iatroscan MK6 device (Iatron laboratories), and chromatograms were integrated using the Chromstar software. Mass detection data were converted in moles using calibration curves established with various

amounts of oleic acid, monoolein, diolein and triolein, used as reference standards. The lipolysis level was expressed as the percentage of free fatty acids (FFA) and monoglycerides present at the digestion end relative to the total amount of acyl chains present in the test meal triglycerides.

2.6. Study of the effects of bioactive compounds on pancreatic lipase activity

The effects of the different bioactive compounds on pancreatic lipase activity were also studied. Enzymatic kinetics were recorded using a pHstat (Metrohm titrino 718, Villebon-sur-Yvette, France) and trioctanoin as substrate. The triglyceride substrate hydrolysis by the lipase was monitored by adding 0.1 N sodium hydroxide (NaOH) when the pH decreased from the set threshold (pH 8). It was considered that the amount of NaOH (µmol) added over time to maintain the pH constant was equivalent to the amount of FFA produced by the lipase activity, if the FFA were completely ionized (pKa < pH). The chosen pH threshold corresponded to the value at which the enzyme exhibits its maximum activity (pH = 8). The enzymatic reaction was performed using 250 µL of trioctanoin dispersed by mechanical stirring in 15 mL of a solution containing 0.3 mM Tris, 2 mM $CaCl_2$, 150 mM NaCl and 0.5 mM or 4 mM sodium taurodeoxycholate. Several concentrations of the bioactive compounds were tested. A stock solution of pancreatin at 10 mg/mL was prepared in pure water, and 3 µL were used for each assay, with 5 µl of colipase (1 mg/ml) that is required to measure the full enzyme activity in the presence of bile salts. Purified bioactive compounds were added during the pHstat test. Three attempts were made to study the inhibitory effect of each compound, and different concentrations of each compound were tested. Their concentrations were chosen to represent those present in pulses, according to previous data (Margier, et al., 2018).

2.7. Statistical analysis

Results are expressed as the mean ± SEM. Differences between more than two groups of unpaired data were tested using the non-parametric Kruskal-Wallis test. The non-parametric Mann-Whitney U test was used as a post-hoc test when the Kruskal-Wallis test showed significant differences among groups. P values ≤ 0.05 were considered significant. All statistical analyses were performed using the GraphPad Prism software, version 8.4.3 (GraphPad Software, San Diego, California, U.S.A.).

3. Results

3.1. Mineral solubility is decreased in meals containing pulses

Iron solubility, expressed as the percentage of soluble iron relative the iron recovered in the digesta, was significantly reduced in meals containing chickpeas compared with meals containing beef (-11.5 ± 1.8% in the chickpea meal, -24.3 ± 0.9% in the beef + chickpea meal and -32.2 ± 1.7% in the semolina + chickpea meal, $p \leq 0.05$) (Fig. 1A). Similar results were obtained concerning zinc and magnesium solubility (-17.0 ± 0.8% in the chickpea meal, -29.6 ± 4.1% in the beef + chickpea meal and -35.6 ± 2.7% in the semolina + chickpea meal; -13.0 ± 1.3% in the chickpea meal, -20.0 ± 0.9% in the beef + chickpea meal and -25.2 ± 3.2% in the semolina + chickpea meal, respectively, $p \leq 0.05$).

Solubility values were lower when solubility was expressed as the percentage of soluble mineral relative to the total mineral initially present in the different test meals, but the decrease observed for the three minerals in the presence of chickpeas remained significant (Fig. 1B).

3.2. Mineral solubility is only slightly decreased by isolated bioactive compounds

Analysis of the effects of isolated bioactive compounds on mineral solubility (Fig. 1C) showed that tannins and saponins significantly decreased iron solubility expressed as the percentage of mineral remaining in the digesta (- 6.0 ± 0.5% and – 9.1 ± 1.0%, respectively, $p \leq 0.05$). Zinc solubility also was significantly decreased in the presence of phytates, tannins and saponins (- 16.5 ± 3.3%; - 25.5 ± 6.8% and - 24.4 ± 1.1%, respectively, $p \leq 0.05$). Magnesium solubility was not modified. When solubility was expressed as the percentage

relative to the mineral initial quantity in the different test meals, only tannins showed a negative impact on iron solubility (- 35.6 ± 0.5%, $p \leq 0.05$) (Fig. 1D).

3.3. Vitamin D stability and bioaccessibility are strongly influenced by the meal composition

Analysis of vitamin D bioaccessibility in the different test meals (Fig. 2) showed that when bioaccessibility was expressed as the percentage of micellar vitamin D relative to the vitamin D present in the whole digesta, vitamin D transfer to micelles was decreased in the presence of chickpeas (- 45.3 ± 3.8% in the beef + chickpea meal, and - 44.5 ± 4.3% in the semolina + chickpea meal, $p \leq 0.05$). However, when bioaccessibility was expressed as the percentage of micellar vitamin D relative to the vitamin D amount initially present in the meals, the presence of semolina and/or chickpeas increased vitamin D bioaccessibility (+ 539.8 ± 110.3% for the semolina meal, + 519.3 ± 45.1% for the chickpea meal, + 180.9 ± 32.0% for the beef + chickpea meal, and + 522.0 ± 27.6% for the semolina + chickpea meal). This discrepancy was due to major differences in vitamin D stability during the *in vitro* digestion process (Table 2).

3.4. Vitamin D bioaccessibility is decreased by phytates and tannins, and increased by saponins

Finally, analysis of the effects of isolated bioactive compounds on vitamin D bioaccessibility showed that vitamin D bioaccessibility, expressed as the percentage of vitamin D recovered in the digesta, was decreased by phytates and tannins (- 28.7 ± 1.4% and - 18.2 ± 2.3%, respectively, $p \leq 0.05$) and was increased by saponins (+ 8.9 ± 4.5%, $p > 0.05$). This was not modified when bioaccessibility was expressed as the percentage of the vitamin D initial amount in the test meal, possibly due to the minor modifications of vitamin D stability (i.e. same test meal) in the different conditions (Table 2).

3.5. Lipolysis is delayed in the presence of chickpeas, partly due to inhibition of the pancreatic lipase activity

Lipolysis rate reached 32.2 ± 3.5% at the end of the *in vitro* digestion of chickpeas, and 47.7 ± 6.5% at the end of the *in vitro* digestion of semolina (data not shown).

Pancreatic lipase activity was then inhibited by addition of different bioactive compounds during the lipolysis reaction (Fig. 3). First, the effects of these compounds were measured when the lipase was in the presence of colipase and 0.5 mM bile salts, the optimum concentration for measuring pancreatic lipase activity. Indeed, higher concentrations could have concealed the inhibitory effects of bioactive substances. Then, the inhibition assays were repeated in the presence of 4 mM bile salts, a micellar concentration that allows displacing surfactants from the oil-water interface. This procedure allows detecting non-specific inhibition due to the surfactant effects of the tested molecules. The results showed that tannins were the strongest inhibitors among the tested bioactive compounds. Indeed, the activity of pancreatic lipase present in pancreatin decreased in a dose-dependent manner upon addition of tannins (from 225 µg to 4 mg per assay; Fig. 3). Moreover, the tannin inhibitory effect observed at low bile salt concentration was still present when the bile salt concentration was increased to 4 mM. This suggested a specific interaction with pancreatic lipase. Lipase activity was reduced by 50% after addition of 2 mg of tannins, which corresponded to an activity of 53.3 U/mg versus 106.7 U/mg for the pancreatin control (1 U=1 µmole FFA released per min). Phytate inhibitory effect was less marked, with a residual activity of 68.8% after addition of 15 mg of phytates. Saponin inhibitory effect decreased when the bile salt concentration was increased to 4 mM (Fig. 3). This suggested that saponin inhibitory effects on pancreatic lipase were only due the surface activity of saponins and not to a specific interaction with the lipase. Finally, cellulose had an extremely weak inhibitory effect with a residual activity of 94%.

Discussion

The aim of this study was to assess the effect of the composition of complex test meals on vitamin D and mineral (iron, zinc and magnesium) bioaccessibility/solubility.

We particularly focused on vitamin D and iron because many people might be at high risk of deficiency across the world. In 2011, 29% of non-pregnant women, 38% of pregnant women, and 43% of children had anemia worldwide (Stevens et al., 2013). Moreover, it has been reported that iron deficiency accounts for 25% and close to 40% of all anemia cases in preschool children and non-pregnant women, respectively (Petry et al., 2016). Even in high income countries, anemia prevalence was about 16% (11% and 22 % in preschool children and non-pregnant women, respectively) (Stevens et al., 2013). Similarly, vitamin D deficiency and insufficiency remain a public health issue in high and low income countries (Holick, 2017). As pulses contain bioactive compounds that can negatively interact with these two families of micronutrients, it is important to investigate whether eating more pulses can exacerbate such insufficiencies.

To this aim, we used an *in vitro* digestion model to simultaneously assess vitamin D bioaccessibility and mineral solubility. *In vit*ro models are useful tools to investigate nutrient, micronutrient and contaminant bioaccessibility (i.e. their transfer to the aqueous absorbable phase of the bolus) (Brodkorb et al., 2019). Our *in vitro* digestion model was previously fully validated for assessing fat-soluble micronutrient bioaccessibility from complex test meals (Goncalves et al., 2013; Reboul et al., 2006). The advantage of this model is the easy solubilization of test meals in high amount of buffer at the beginning of the digestion. Although less representative of the physiological conditions compared to the INFOGEST procedure (Brodkorb et al., 2019), this does not affect the model ability to predict fat-soluble micronutrient bioavailability in a healthy human population (Reboul et al., 2006). Moreover,

this model is easy to set up and was miniaturized in this study by dividing by two all the product quantities compared with the original protocol (Goncalves et al., 2013).

Our findings first confirmed that the presence of chickpeas in a meal can alter iron, zinc and magnesium solubility. Solubility is required for the possible mineral intestinal absorption and is a criterion used to assess mineral availability (Arpadjan, Momchilova, Venelinov, Blagoeva, & Nikolova, 2013; Engle-Stone, Yeung, Welch, & Glahn, 2005). The most important effects were observed in test meals containing only semolina and chickpeas. Overall, this confirms that mineral bioavailability is reduced in plant-based meals (Gibson, Heath, & Szymlek-Gay, 2014), and indicates that the presence of pulses, even in a meal containing beef, is deleterious. Among the different bioactive substances present in pulses, tannins (at concentrations mimicking those found in chickpeas) were the most efficient mineral chelators, especially iron. This is in agreement with previous data in humans showing that in the absence of calcium, tannic acid but not phytic acid decreases the fasting non-heme iron bioavailability (Jaramillo et al., 2015). This is also consistent with the observation that tannic acid-iron complexes are less soluble and more stable than phytic acid-iron complexes (Engle-Stone et al., 2005).

Our results also showed that when expressed as the percentage of vitamin D recovered in the digesta at the end of the digestion protocol, chickpeas in a meal can limit vitamin D transfer to the micellar phase of the bolus. These results confirm our previous work showing that fat-soluble vitamin bioaccessibility is significantly lower in test meals made of meat and pulses than in test meals made of meat and potatoes (Margier et al., 2019). These data are supported also by the results of the lipolysis analysis. Indeed, we found that the lipolysis rate was lower in the meals containing chickpeas than in the meals with only semolina. The lower concentration of lipolysis products (FFA, monoglycerides) in the micellar phase obtained during the digestion of the chickpea meal could results in decreased vitamin D incorporation in mixed micelles, and consequently in reduced bioaccessibility. However, results were quite

different when vitamin D bioaccessibility was expressed as the percentage of vitamin D incorporated in the test meal. In this case, vitamin D bioaccessibility was significantly lower in the test meal containing beef than in test meals containing plant-based food only. This was due to the important degradation of vitamin D during *in vitro* digestion of meals containing beef, and/or higher protection against degradation in plant-based meals. Indeed, it is acknowledge that meat is a prooxidant factor during digestion, and can lead to the generation of reactive oxygen species and lipid oxidation products (Van Hecke, Van Camp, & De Smet, 2017). Conversely, pulses and cereals contain several antioxidants, such as phenolic compounds (Durazzo, Casale, Melini, Maiani, & Acquistucci, 2015; Margier, et al., 2018), that can protect vitamin D. However, vitamin D degradation during *in vivo* digestion is likely less important than during *in vitro* digestion where vitamin D is more exposed to UV and oxygen. Additional studies using *in vivo* approaches are needed to determine which factor (i.e. increased transfer vs increased degradation in the presence of beef) is preponderant in modulating vitamin D bioaccessibility. In standardized test meals with only potatoes , in which vitamin D degradation was similar in the different conditions, vitamin D bioaccessibility was decreased by addition of purified phytates and tannins (two bioactive compounds normally found in pulses), in accordance with our previous results for vitamin K (Margier et al., 2019). Conversely, and unlike vitamin K, vitamin D bioaccessibility was increased by saponins that have cyclic triterpenoid or steroid structures. Saponin surfactant properties (Ruyssen, 1946) may contribute to vitamin D solubilization. This discrepancy is probably related to the very different molecular structures of vitamin K (a naphthoquinone) and vitamin D (a seco-steroid). We might hypothesize that saponins compete with vitamin K for incorporation in the mixed micelles, while they may associate with vitamin D and thus increase its solubility in the aqueous phase. This is in line with a previous study showing vitamin D self-association and thus solubilization in the aqueous phase at high concentrations (Desmarchelier et al., 2018).

Interestingly, the experiments with pancreatic lipase showed that when used at concentrations similar to those found in pulses, purified bioactive compounds can also display an inhibitory effect on lipase activity. This observation might explain the decrease in vitamin D bioaccessibility. Analysis of the effects of bioactive substances on the lipase activity in the presence of colipase and 0.5 mM bile salts and then 4 mM bile salts (a more physiological concentration but that could suppress non-specific inhibition by surfactant molecule) showed that tannins had the most important inhibitory effect. This could be due to the formation of complexes with pancreatic lipase, as demonstrated for other enzymes (Rocchetti, Giuberti, Gallo, Bernardi, Marocco, & Lucini, 2018). It was previously reported that saponins bind specifically to pancreatic lipase and inhibit its activity (Zhao, Sim, Shim, Ha, Kang, & Kim, 2005). Saponins displayed some inhibitory effect on pancreatic lipase activity in the experiments with low bile salt concentration, but this effect was decreased in the presence of 4 mM bile salts, suggesting that this inhibition is not the result of a specific interaction with the lipase. Phytates displayed a moderate inhibitory action compared with tannins. Previous studies showed that phytates interfere with the activity of the pancreatic lipase (REF), but the underlying mechanisms remain unclear. Finally, cellulose, used as a fiber model, had a minor inhibitory effect. We now need to evaluate the effects of the combination of these compounds because they may have additive or antagonistic effects (Margier et al., 2019).

Overall, we demonstrated that our *in vitro* digestion model is suitable for assessing the bioaccessibility/solubility of minerals and fat-soluble micronutrients. We found that complex meals made only of plant-based products may lead to reduced micronutrient bioaccessibility. Therefore, this *in vitro* digestion model could be particularly useful for testing different recipes in order to improve micronutrient bioavailability by using other food products (Platel & Srinivasan, 2016). However, additional studies are needed to validate these findings i)

using an enterocyte culture model to assess the effect of the different test meals on absorption, and ii) using *in vivo* models to fully evaluate micronutrient bioavailability.

Once confirmed, these data will help to better integrate pulses in a balanced diet, for example by associating them with micronutrient-rich foods or with foods that promote micronutrient bioavailability. These findings mays also be used by manufacturers to optimize the nutritional quality of recipes containing pulses.

Conflict of interest:

Other authors declare no conflicts of interest or financial interest.

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Figure legends

Figure 1: Iron, zinc and magnesium solubility

A-C: Mineral solubility expressed as the percentage of the indicated minerals found in the digesta.

B-D: Mineral solubility expressed as the percentage of the indicated minerals in the test meals.

Test meals were constituted of beef and/or semolina and/or chickpeas (A-B), or of pureed potatoes enriched with purified cellulose (0.7mg), phytates (42.5 mg), saponins (6 mg) and tannins (0.4 mg). (C-D). *In vitro* digestion was performed to mimic the oral, gastric and duodenal stages. Data are the mean ± SEM, n = 4. * $p \leq 0.05$ when compared with the "beef" test meal.

Figure 2: Vitamin D bioaccessibility

A-C: Vitamin D bioaccessibility expressed as the percentage of vitamin D found in the digesta.

B-D: Vitamin D bioaccessibility expressed as a percentage of vitamin D present in the test meals.

Test meals were constituted of beef and/or semolina and/or chickpeas (A-B), or of pureed potatoes enriched with purified cellulose (0.7mg), phytates (42.5 mg), saponins (6 mg) and tannins (0.4 mg). (C-D). *In vitro* digestion was performed to mimic the oral, gastric and duodenal stages. Data are the mean ± SEM, n = 4. * $p \leq 0.05$ when compared with the "beef" test meal.

Figure 3: Effects of bioactive compounds on pancreatic lipase activity.

Lipase activity was measured in the presence of 0.5 mM or of 4 mM bile salts, and the residual activity after addition of the bioactive compounds was expressed as percentage of the activity measured with pancreatin alone. Various amounts of bioactive compounds were tested: tannins (225 µg, 400 µg, 2 mg, 4 mg), phytates (1.5 mg, 15 mg), saponins (1 mg, 2 mg), and cellulose (100 mg). Data are representative of at least 3 experiments.

Tables

Table 1: Bioactive compound and fiber content of chickpeas, semolina and potatoes

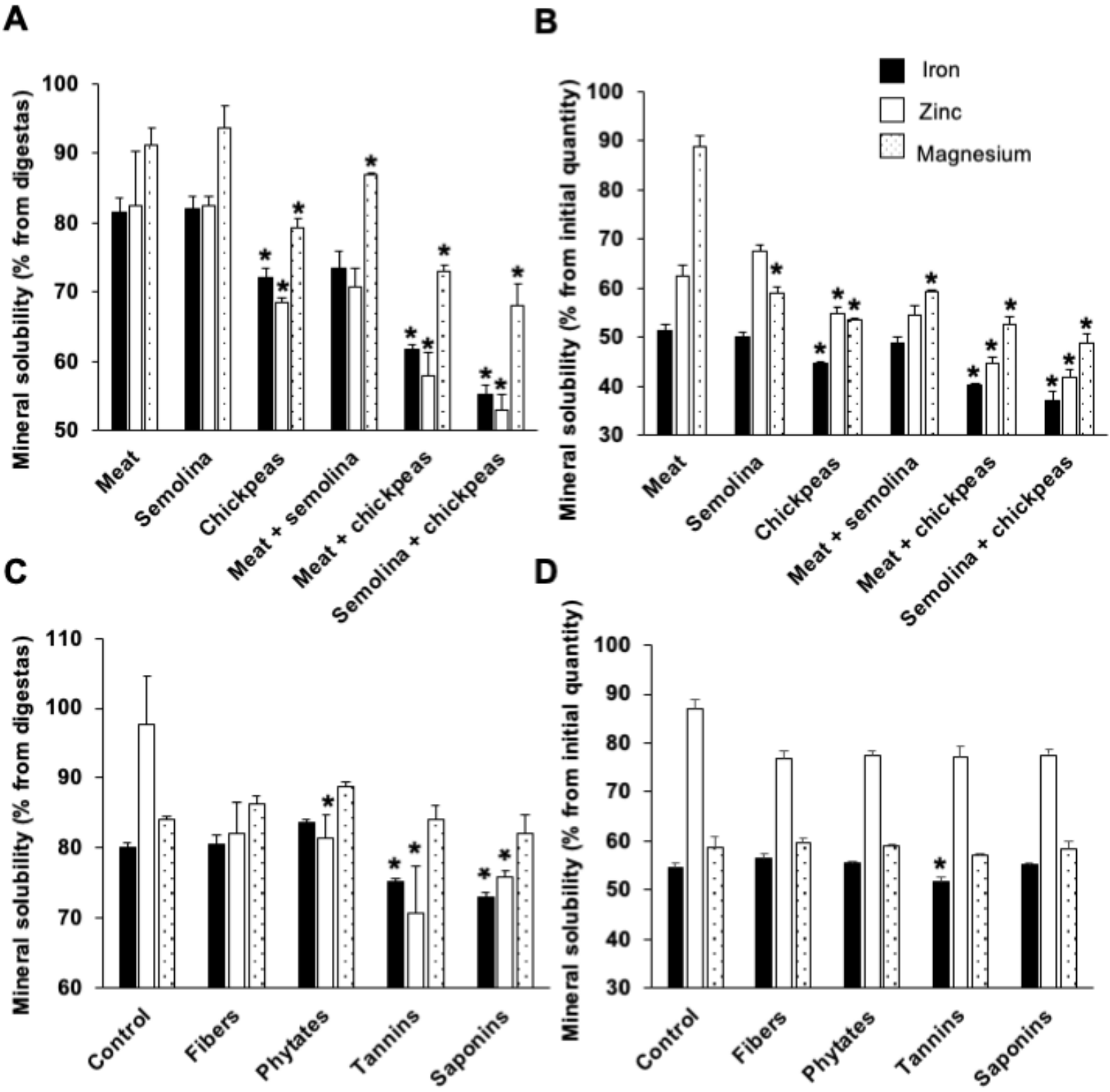
	Bioactive substances			
	Fibers (g/ 100 g)	**Phytates (mg/100g)**	**Saponins (mg/100g)**	**Tannins (mg/100g)**
Pureed potatoes	2.2 ± 0.0 [a]	141.5 ± 19.6 [a]	32.3 ± 1.3 [a]	0 (Not detectable) [a]
Cooked semolina	1.6 ± 0.2 [a]	518.0 ± 25.9 [b]	16.0 ± 1.6 [b]	7.45 ± 1.9 [b]
Canned chickpeas	6.4 ± 2.1 [b]	526.4 ± 22.3 [b]	116.8 ± 0.2 [c]	13.7 ± 1.5 [c]

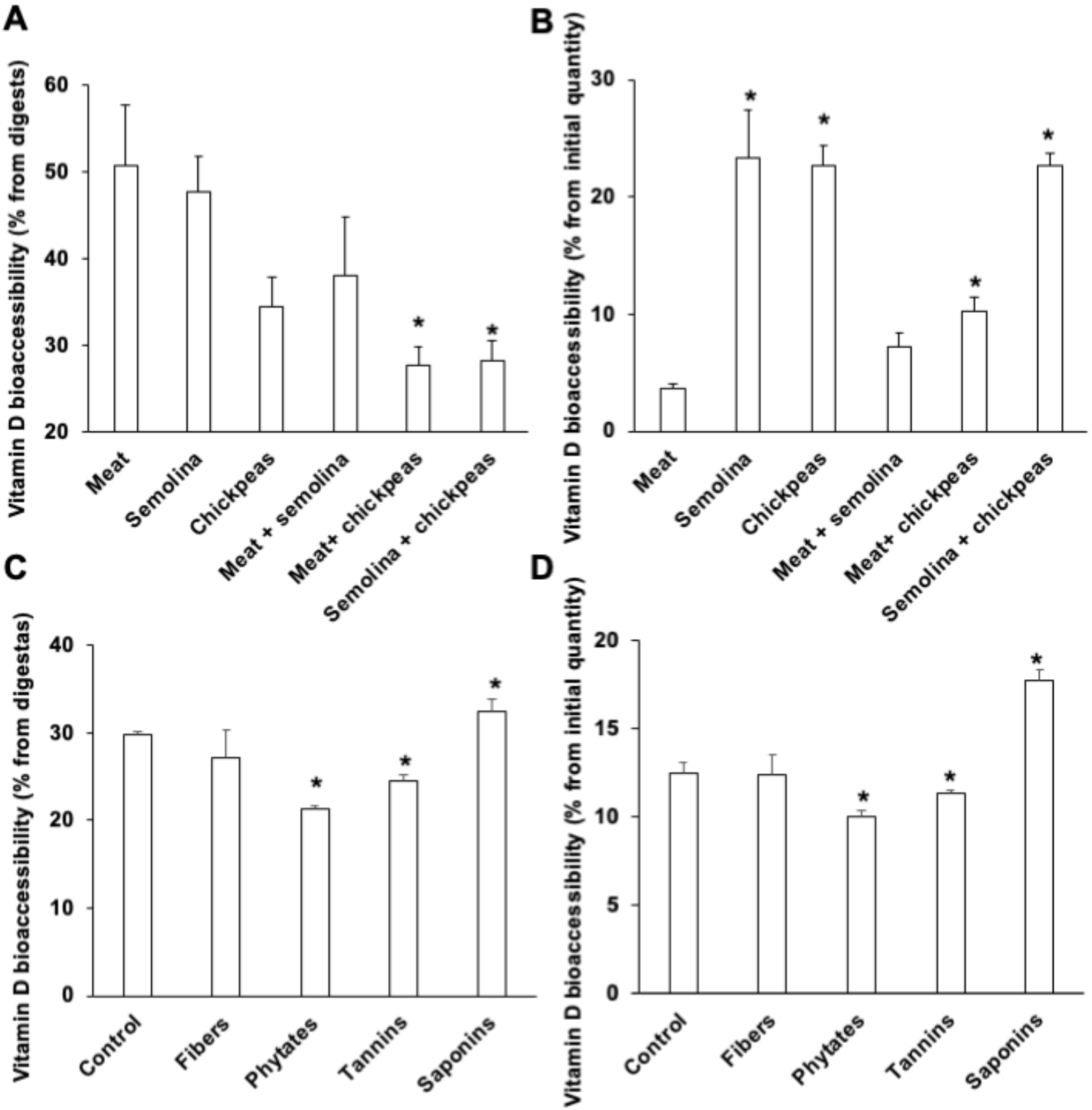
Different superscript letters in the same column indicate a significant difference between groups ($p \leq 0.05$).

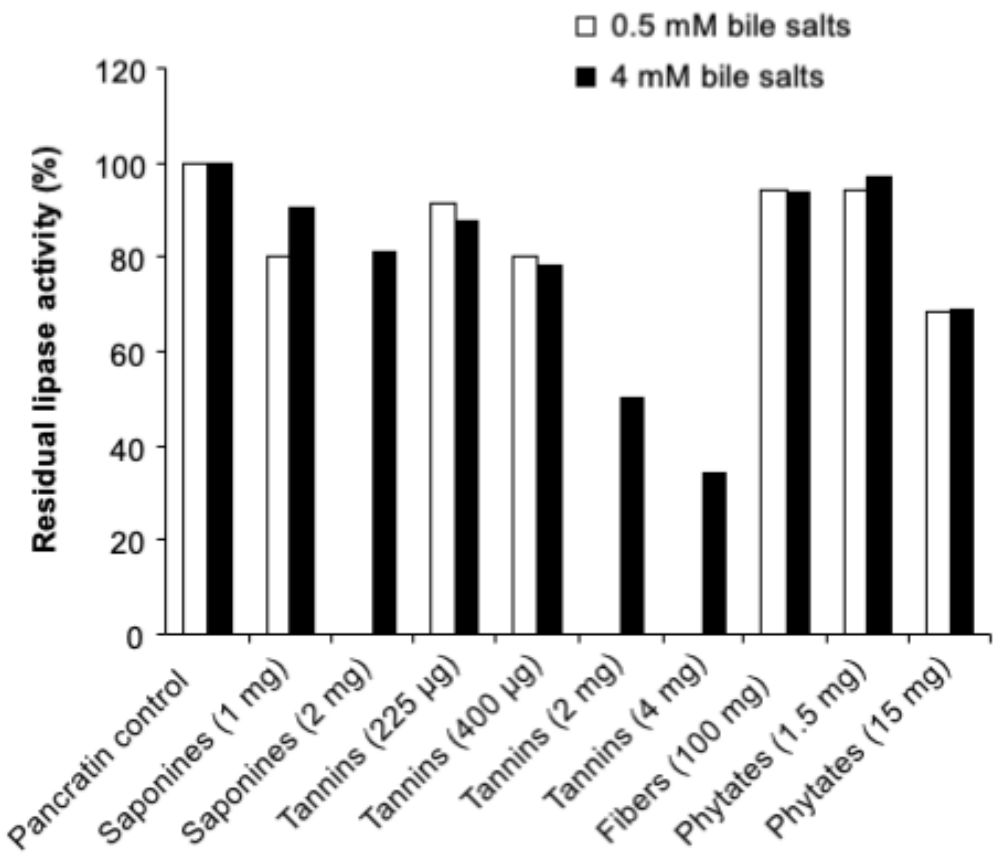
Table 2: Remaining vitamin D at the end of the *in vitro* digestion procedure

Test meals	% of remaining vitamin D
Beef	8.4 ± 1.6 [a]
Semolina	52.6 ± 11.0 [b, d]
Chickpeas	66.8 ± 3.6 [b, c]
Beef + semolina	22.8 ± 3.1 [b, a]
Beef + chickpeas	38.4 ± 5.1 [a, b, e]
Chickpeas + semolina	83.6 ± 4.5 [c, d, e]
Potatoes	41.5 ± 3.2 [a]
Potatoes + fibers	46.0 ± 2.4 [a]
Potatoes + phytates	53.8 ± 6.7 [a]
Potatoes + tannins	53.7 ± 5.8 [a]
Potatoes + saponins	54.9 ± 2.4 [a]

Remaining vitamin D was the percentage of the vitamin D amount in the digesta at the end of the *in vitro* digestion procedure relative to the vitamin D amount initially added to the test meals (n = 3). Different superscript letters in the same table cell indicate a significant difference between groups ($p \leq 0.05$).


A
Mineral solubility (% from digestas)
100
90
80
70
60
50
Meat
Semolina
Chickpeas
Meat + semolina
Meat + chickpeas
Semolina + chickpeas
B
Iron
Zinc
Magnesium
Mineral solubility (% from initial quantity)
100
90
80
70
60
50
40
30
Meat
Semolina
Chickpeas
Meat + semolina
Meat + chickpeas
Semolina + chickpeas
C
Mineral solubility (% from digestas)
110
100
90
80
70
60
Control
Fibers
Phytates
Tannins
Saponins
D
Mineral solubility (% from initial quantity)
100
90
80
70
60
50
40
30
Control
Fibers
Phytates
Tannins
Saponins


A
Vitamin D bioaccessibility (% from digests)
60
50
40
30
20
Meat
Semolina
Chickpeas
Meat + semolina
Meat+ chickpeas
Semolina + chickpeas
B
Vitamin D bioaccessibility (% from initial quantity)
30
20
10
0
Meat
Semolina
Chickpeas
Meat + semolina
Meat+ chickpeas
Semolina + chickpeas
C
Vitamin D bioaccessibility (% from digestas)
40
30
20
10
0
Control
Fibers
Phytates
Tannins
Saponins
D
Vitamin D bioaccessibility (% from initial quantity)
20
15
10
5
0
Control
Fibers
Phytates
Tannins
Saponins


0.5 mM bile salts
4 mM bile salts
Residual lipase activity (%)
120
100
80
60
40
20
0
Pancratin control
Saponines (1 mg)
Saponines (2 mg)
Tannins (225 µg)
Tannins (400 µg)
Tannins (2 mg)
Tannins (4 mg)
Fibers (100 mg)
Phytates (1.5 mg)
Phytates (15 mg)